SHORT-TERM PHOTIC REGULATION OF A RECEPTOR MECHANISM IN A DINOFLAGELLATE[1]

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The directional response of organisms with respect to a light stimulus has been under investigation for over a century. Study of the phototaxis of unicellular organisms was initiated by Cohn (1865), who noted that many microörganisms respond to blue light by a change in swimming direction with a subsequent orientation toward the light source. However, in spite of the voluminous literature on the subject now at hand (*Vide* Haupt, 1959; Bendix, 1960; Halldal, 1964; Jahn and Bovee, 1967), advances in the understanding of the mechanisms by which this process takes place are still few and unrelated. There exists great variability in response from one form to the next, and even within a given species. Several investigators have noted extremes of responsiveness, depending upon the age of the culture (Halldal, 1956), the time of day (Bruce and Pittendrigh, 1956), and even such factors as ion balance and pH (Halldal, 1959).

It is obvious that the phototactic process involves much more than a simple light-to-photoreceptor-to-"motorium" relationship. The regulating mechanisms involved must be elucidated and their relationship to the photoactive machinery determined if the consequence of phototaxis with respect to the ecology of the organism is to be understood.

It is the purpose of this report to give evidence for a photoregulatory system and its possible relation to the receptor-effector chain in the phototactic response of the dinoflagellate *Gyrodinium dorsum* Kofoid.

MATERIAL AND METHODS

Culture methods

Cultures of *Gyrodinium* were grown in a sea-water-based medium as previously described (Hand, Collard and Davenport, 1965). The cultures were maintained in a light- and temperature-controlled environment, the light being furnished from above by eight 20W daylight fluorescent tubes and a 60W incandescent lamp. The total illumination at the surface of the culture was approximately 400 foot-candles. The light was cycled to give a 16–8 light-dark daily period. Temperature in the culture box was maintained at 20° C. Under these conditions cultures came to peak growth (approximately 10,000 cells/cc.) in two weeks. Cultures from 4 to 5 days old were selected for experimentation because of their high degree of responsiveness; cultures one week or more old were found to give a generally poor light response. All experiments were conducted between the fourth and sixth hour of the light phase (9–11 AM). Room temperature was maintained at 20° C.

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Apparatus

Experiments were conducted using the flying-spot scanning apparatus previously described (Davenport, Wright and Causley, 1962; Hand *et al.*, 1965). Its operation may be briefly described as follows: An oscilloscope tube generates a square sweep of low intensity light. This scan passes through the sample and is perceived by a photo-multiplier tube. An organism intercepting the scanning beam is reported as a potential change by the photocell. This potential change is ultimately displayed on a viewing readout oscilloscope as an image of the organism, giving its size and position. In effect, the image seen is the "shadow" cast by the organism.

By coupling a Dumont type 450 oscilloscope camera fitted with a Robot "Recorder 24" 35mm. camera-back to the display scope, the images of moving organisms may be photographically recorded on Tri-X Pan film as they occur. Hence, in a single picture of the display screen, one can obtain data from a number of organisms for linear velocity, rate of change of direction, aggregation, and orientation (the type of data gathered being dependent on scan rate and film exposure). In some experiments, where a running sequence of events was desired, a Bolex H16 Rex III motion picture camera was used to collect the data.

For the experiments to be described, the following additions were made to the basic apparatus. The organisms being scanned were stimulated by light from a Bausch and Lomb No. 33-86-02 Grating Monochromator. This instrument was adjusted to give a 20 mμ band-pass between 400 and 700 mμ. Intensity was controlled with an Optical Coating Laboratory continuously graded neutral density filter. Stimulus duration and interval were controlled by a simple revolving blanking wheel in which a notch of the required size was cut. The wheel was driven by a Bodine type NSH 128 motor. Wheel velocity was controlled by a rheostat. A stimulus marker was operated in conjunction with the revolving disc.

In some experiments, a second red light source was used. Its beam entered the preparation under study at a 90° angle to the axis of the stimulus beam from the monochromator. The light source consisted of a Toyoda microscope lamp. Its intensity was controlled by the associated Toyoda rheostat. The wavelengths of this source were controlled with a Wratten A No. 25 glass filter, having a low spectral cut-off at approximately 580 mμ. This filter allowed essentially all the energy in the light source between 600 and 700 mμ to pass.

Because the Toyoda lamp produced a very hot emission, a "heat sink" was introduced between the light source and the sample. This consisted of a 20-cm. Plexiglas tube filled with distilled water. Under these conditions the red light produces only a 1.0° C. temperature change after 30 minutes illumination at the scanning site.

Responses and data collection

Two responses were studied: the "positive" phototactic or migratory response and what we have called the "stop-response." Under the conditions of our experiments a "negative" phototaxis has never been observed. The stop-response has been observed in many forms and was given the name "shock reaction" by Engelmann (1879). It consists of a rapid cessation of movement following a brief but intense light stimulus. As will be seen, this response provides an excellent tool for the study of the photoreceptor machinery, because of its all-or-none nature.

For the investigation of responses cells were placed on the microscope stage in a specially designed microscope slide (Fig. 1), in which the total cell preparation could be subjected to light from one or both sources.

Data recording was accomplished by photographing the appropriate response. The method of data collection depended on the kind of experiment being conducted, and consisted of counts of cells, comparisons of numbers of cells moving with numbers of cells not moving, and orientation of the cells with respect to a given light source. In experiments where a motion picture camera was used, a frame-by-frame analysis of the film was performed to obtain the data.

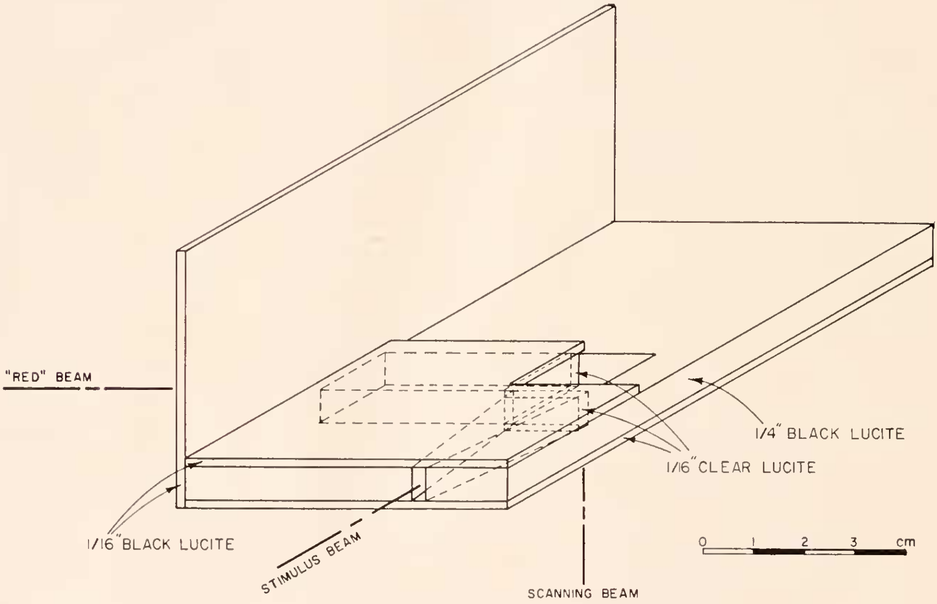

Figure 1. The preparation slide. The cell suspension is contained in the center well. The axes of the light beams intersect in the center well through clear Plexiglas windows.

Experiments

I. *The action spectrum of phototaxis*

Positive phototaxis in *Gyrodinium* consists of a migration of cells toward the stimulus source, resulting eventually in the accumulation of individuals at the wall of the slide-well through which the stimulus light enters the well. This area is scanned, and a photograph is taken at the time the stimulus is introduced and again after the cells have been illuminated for one minute. The total number of cells in each case may be counted and compared, with the resulting ratio giving the indication of phototactic activity. As a control a series of tests was run in which no stimulus was given. In this series the possible effects of raster illumination or of irregular distribution by the initial pipetting were eliminated.

For a single-beam action spectrum determination of this kind to be correct, wavelengths must be of equal energy. To accomplish the energy equilibration, the monochromator was calibrated using an Eppley vacuum thermopile. Readout from the thermopile was taken with a Keithley Model 149 Mill-Microvolt Meter. The light from the monochromator was passed through the neutral density filter and a plate of Lucite before being intercepted by the thermopile. This was done to insure that the resulting energy measurement was equal to that which the cells would encounter under experimental conditions. The initial calibration adjusted all energies from 420 mμ through 550 mμ to the maximum energy at 420 mμ. A phototactic response curve was determined at this energy in a point-by-point manner. A 0.6-ml. aliquot was taken from the culture, and placed in the slide well. The resulting preparation was covered with a standard glass coverslip, removed from the culture chamber and placed on the scanning stage in the dark. It was allowed to stand undisturbed for three minutes before being tested for the response, this time being necessary to allow the cells to reach random distribution in the well. The sample was then stimulated in the prescribed manner and following photography was discarded. Several samples were taken at each wavelength to insure an adequate sample size (n = 100). The resulting action spectrum may be seen in Figure 2.

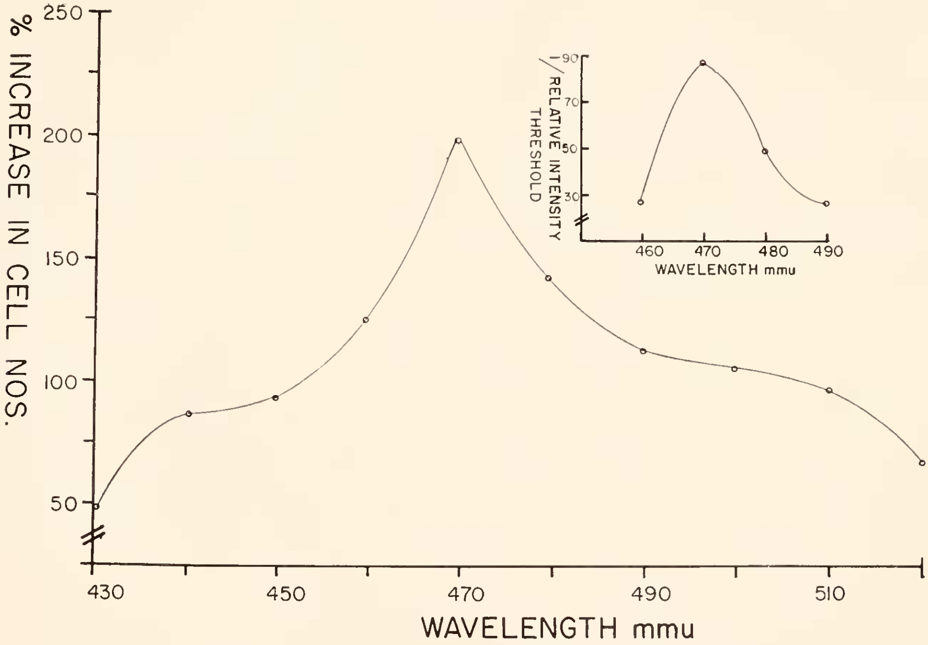

FIGURE 2. The action spectrum for positive phototaxis as determined by an equal-energies method. The points determining the curve represent the maximum increase in cell numbers (accumulation) following a given stimulus. Inset: The action spectrum for the active spectral region of positive phototaxis. The points determining the curve represent the lowest relative intensity at which maximum orientation was observed during a 10-second stimulation.

In order to determine as accurately as possible the "peak area" of this spectrum, the region between 460 and 490 mμ was re-examined using a threshold determination method. The intensity threshold was defined in this series of experiments as the intensity which elicited maximum orientation during a 10-second illumination. The prepared sample was allowed to remain in darkness for 3 minutes, then a 10-second light stimulus of a given intensity and wavelength was administered. A 2-second camera exposure recorded the orientation of the cells with respect to the stimulus direction. This process was repeated, each time with a new sample, until an intensity was reached in which no significant orientation could be observed. Cell pathways exhibiting orientation within 10° of the axis of the stimulus source were compared to the total number of cells present. The resulting ratio indicated the cell sensitivity to the tested wavelength and intensity. The threshold values were corrected for the initial energy differences present in the stimulus source and plotted (Figure 2, inset).

The action spectrum determined for phototaxis in *Gyrodinium* indicates that a single peak for orientation and migration exists at 470 mμ. Because of the wide band-pass of the monochromator we must add an error factor of ± 10 mμ to this determination. A peak around 470 mμ is consistent with the findings of other investigators for dinoflagellates. Halldal (1956) reported that the dinoflagellates *Gonyaulax polyedra* and *Peridinium trochoideum* gave a peak phototactic response at 475 ± 7 mμ.

II. *Some characteristics of the stop-response*

Motion pictures (16 f.p.s.) were made of the stop-response resulting from a 470 mμ light stimulus of 10 seconds duration. Each frame was projected on to a paper screen. The position of each cell in the frame was marked, and the next frame projected and the cells marked. The resulting sets of marks formed the pathway of the cells just prior to and during the stimulus period.

A response latency was calculated, based on the number of position changes following the initiation of the stimulus. These calculations yielded a response *latency* of 400 to 600 msec. Furthermore, it was determined that the response *duration* was 4 to 6 seconds.

In any sample of these cells, a certain amount of stopping occurs without external stimulation. This response is most often caused by encounters between cells, but can occur without any visible external factors. We term this response "random stopping," and in the process of running other experiments, we have found that it can occur in as many as 30% of the cells present during a given period of time. An average value for a sample size of 100 individuals taken from an early (5-day) culture is 14%.

The motion pictures were also examined for the direction of movement taken by the cells following the stop-response. In 50 cells examined, 39 migrated directly toward the stimulus source. The remainder of the cells chose more devious routes, but eventually exhibited a positive phototactic response.

The pathways of cells which failed to stop during a light stimulus were also examined. It was found that these cells failed to respond to the stimulus in any way during the stimulus exposure.

III. *The action spectrum of the stop-response*

If the stop-response is indeed the result of the same photo-machinery responsible for the phototaxis, then both phenomena should have similar characteristics with respect to wavelength sensitivities. To test this, an action spectrum determination was conducted on the stop-response and compared to that previously obtained for the phototactic response.

The action spectrum for the stop-response was also obtained using a threshold determination method. Following a one-minute equilibrating period in the dark, the prepared sample was given a stimulus of 2 seconds at the maximum intensity obtainable for that wavelength. The response was recorded using the still camera at a $\frac{1}{4}$-second exposure, one second after the initiation of the stimulus. Following stimulation, the cells were allowed to remain in darkness for 20 seconds; then they were again stimulated at a lower intensity. This process was repeated until an intensity was reached at which no stop-response above random could be recorded. The wavelength band examined was between 430 and 530 mμ. As in the orientation experiments, the samples were run at 10 mμ intervals, and enough samples were tested to record the responses of at least 100 individuals.

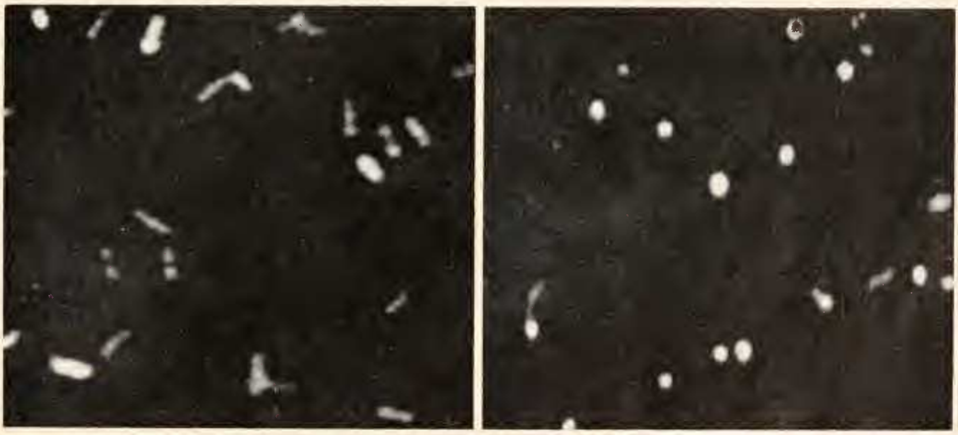

FIGURE 3. One-quarter-second exposures. Left: cells in motion. Right: stop response.

"Threshold" was defined as the intensity at which 50% stopping was obtained. The number of cells stopping was compared to the total number of cells present in the sample to obtain the per cent stopping value. Stopped cells appear on the film as dark circular to ovoid spots, while cells moving appear as less dense rod-shaped marks. This is shown in Figure 3. The values resulting from these counts were corrected with respect to the original monochromator energies and plotted. The resulting action spectrum curve is given in Figure 4.

In examining the action spectrum of the stop-response, it is apparent that the peak coincides with that obtained for phototaxis. On the basis of these results it would appear that the same pigment system operates in both responses. This assumption is not unjustified if one considers the findings concerning the direction of cell movement following the stop-response reported in Experiment II.

As can be seen by comparing the curves for the two responses, the phototactic response threshold is much lower than that obtained for the stop-response. This threshold difference gives some indication as to why the stop-response is generally followed by a migratory response.

IV. *Dark-adaptation of the stop-response*

If the photoreceptor of this organism is related in function to that of an animal photoreceptor, then it should show similar properties with respect to irritability. One quantitative relation between photic stimulation and response magnitude is described by the reversible reaction of light- and dark-adaptation. If a receptor is presented with high intensity stimulation for an extended period, the sensitivity of the receptor falls. If a receptor in this "light-adapted" state is put into darkness, and its sensitivity monitored, the characteristic dark-adaptation curve may be recorded. This curve represents a rise in sensitivity presumably due to the regeneration of a photoactive pigment. In many photoreceptor systems such a curve has been demonstrated. Where the curve involves only a single pigment it has a characteristic monophasic shape.

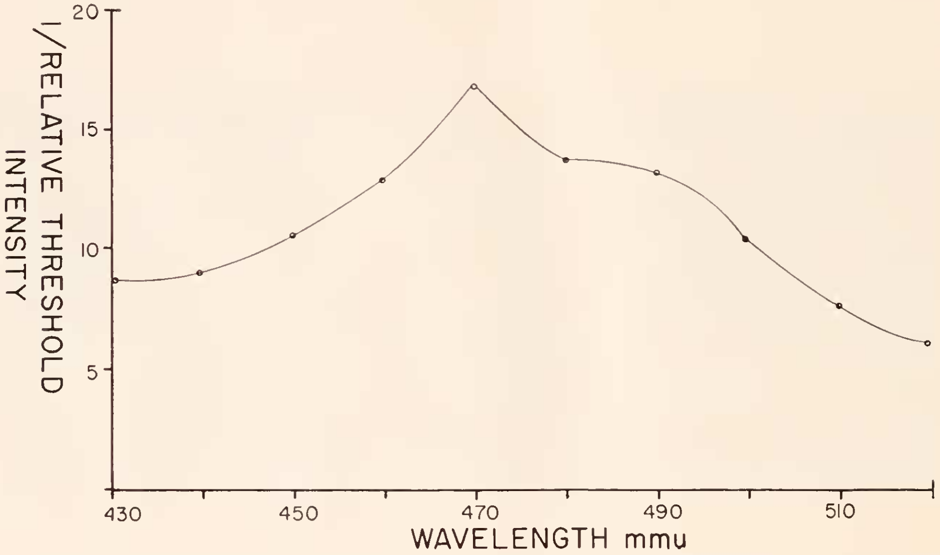

FIGURE 4. The action spectrum of the stop-response as determined by a wavelength-threshold method. The points determining this curve represent the relative intensity per wavelength at which 50% stopping was obtained (n = > 100).

In the experiments described below, the dark-adaptation curve for the stop-response is determined.

Light-adaptation was accomplished by bathing the cells in a 1000-foot-candle tungsten white light for 5 minutes. Following this the cells were put in darkness and stimulated at a given intensity for 2 seconds at 15-second intervals until the cells returned to maximum sensitivity. The response to each stimulus was recorded with the still camera using a ¼-second exposure, given one second after the initiation of the stimulus. The sample was discarded, a new one prepared, light-adapted as before, and then placed in darkness and stimulated at a lower intensity. This process was repeated until the stimulus intensity administered would not elicit any response in 10 minutes. Several samples were taken at each stimulus level. A second series of experiments was performed in which the light-adaptation time was extended to 10 minutes. The results of both experiments may be seen in Figure 5. As the results indicate, the 10-minute light-adapting time gave the same results as the 5-minute time. This indicates that 5 minutes at this intensity is sufficient to "bleach" the photoreceptor pigment completely.

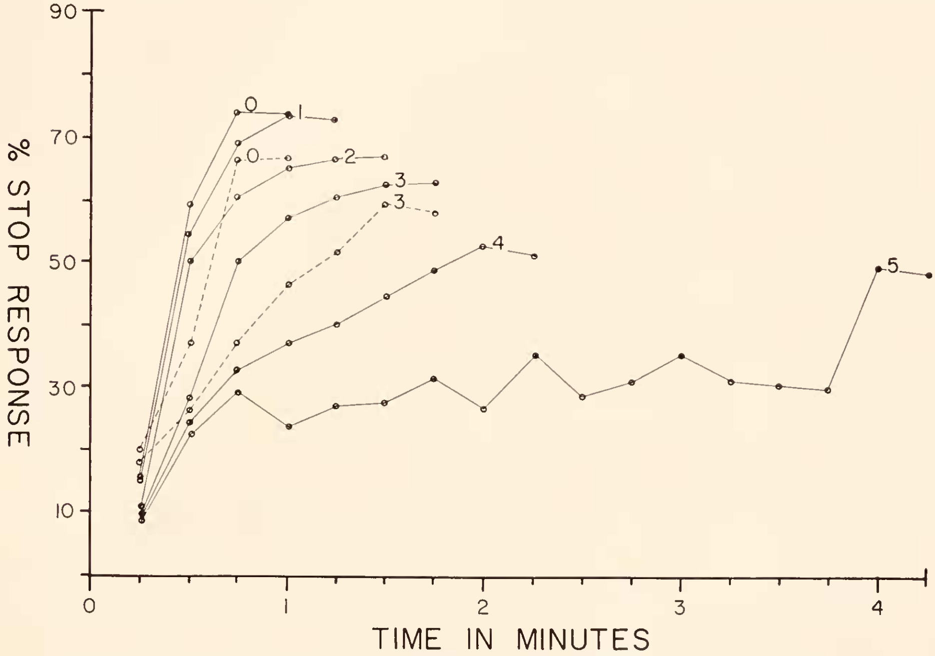

FIGURE 5. The relation of stimulus intensity to stop-response magnitude when light-adapted cells are placed in darkness. The points represent the return to maximum response shown by 100 cells, when these cells are stimulated at different intensities. The numbers represent the different stimulus intensity levels, with 0 = 280 ft.-c., 1 = 213 ft.-c., 2 = 170 ft.-c., 3 = 136 ft.-c., 4 = 113 ft.-c., 5 = 89 ft.-c.

The dark-adaptation curve (Fig. 6, I) was derived from Figure 5. The points determining the curve represent the time at which maximum response was reached at that particular stimulus intensity. It may be argued that the points best representing a threshold value are those points at which the maximum increase in response occurred at the various test intensities. Yet it must be emphasized that the points making up this distribution are based on a population value, and not on any individual. Hence, the maximum response value, as chosen, most closely represents the time in which the largest number of cells in the population regain sensitivity to a given stimulus.

As can be seen, the dark-adaptation curve determined for this behavioral response of *Gyrodinium* resembles in form that obtained by Hartline and McDonald (1947) for single isolated photoreceptors of the horseshoe crab, *Limulus*. This resemblance leads us to believe that the response we are measuring in *Gyrodinium* does directly reflect the nature of photoreceptor action, and that this action involves a regenerative pigment.

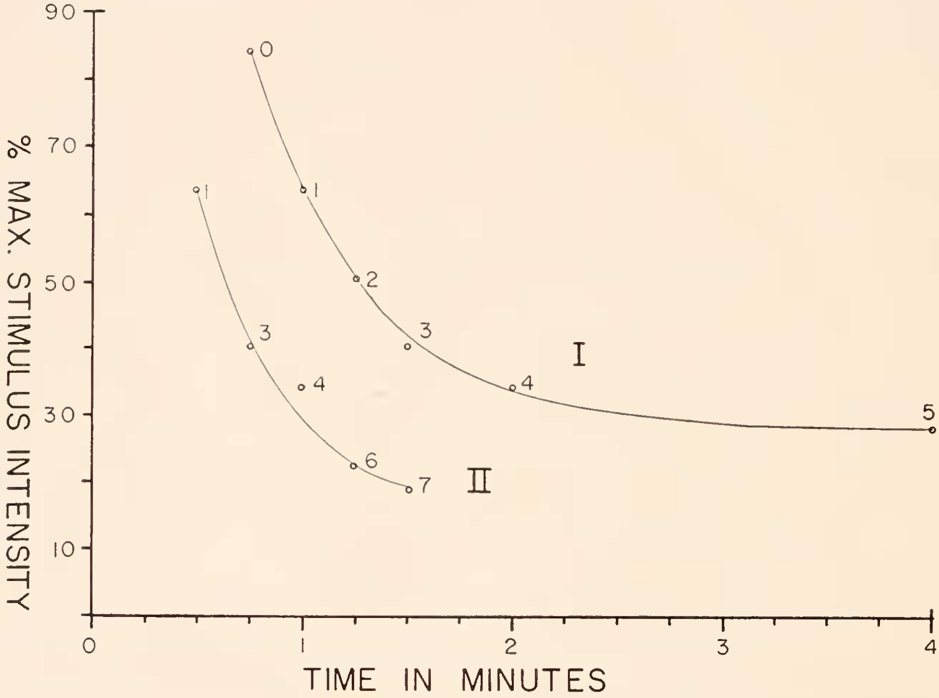

FIGURE 6. (I) The dark-adaptation curve for *Gyrodinium*. The points determining the curve represent the time at which the maximum number of cells responded to each (numbered) stimulus level. (II) The dark-adaptation curve for *Gyrodinium*, when red light is substituted for darkness following light-adaptation.

V. *The effects of darkness and red light on the stop-response*

In early work with *Gyrodinium* we observed that the cells, when placed in darkness for an extended period of time, did not retain their ability to give an orientation or stop-response but did maintain normal motility. A series of experiments were designed to determine the length of time the cells could give a maximum stop-response after being placed in the dark. Samples were placed in darkness and stimulated for 2 seconds at one-minute intervals, using maximum energy at 470 mμ as the stimulus. As a control against the possibility that repetitive stimulation adapted out the response, a second sample was tested in which the cells were stimulated at time 0 and at time 10 minutes. The resulting curve is shown in Figure 7. As can be seen, the response diminishes to one-half of its original value in 10 minutes. The response maintains a high normal value for approximately 6 minutes before degrading. This fact became most important in the design of all experiments described in this paper, in that the total time of testing was kept at or below 5 minutes.

When experiments were begun to test the wavelength sensitivity of the photoreceptor system, it was observed that red light (betwen 600 and 700 mμ) had a profound effect on the response decay phenomenon, but did not elicit any overt cell response. The effect of red light was examined by placing the cells in red light rather than in darkness and stimulating them at one-minute intervals as before.

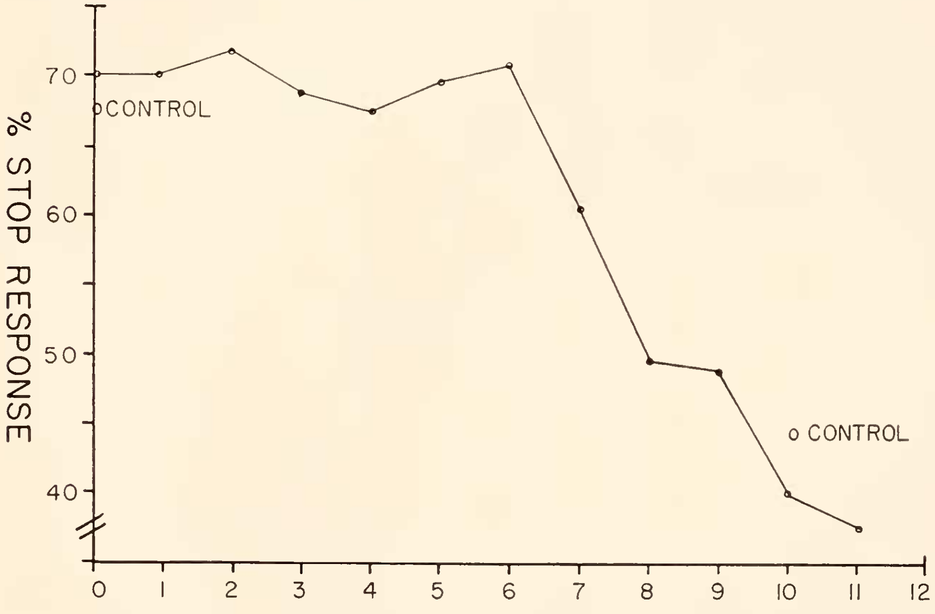

FIGURE 7. The loss of stop-response when cells (n = 100) are placed in darkness. The stimulus is a 280 ft.-c. 470 mμ light of 2 seconds duration, given every minute. The controls represent a stimulus given at 10-minute intervals.

There was extreme variability in the total time the stop-response could be maintained from sample to sample, but it was determined in all cases that cells would maintain maximum sensitivity to the blue stimulus for 15 minutes.

A series of experiments was then designed to define more clearly the relationship between the red- and blue-absorbing pigment systems. The cells were subjected to given combinations of red light, blue light, and darkness, and stimulated with maximum intensity 470 mμ light (280 ft.-c.) for 2 seconds at five-minute intervals. The total time of the experiment was 15 minutes. In cases where blue light was to bathe the cells, this light was maximum intensity 470 mμ light from the monochromator. To effect a true stimulus with this source, the bathing light was interrupted for 15 seconds, thereby placing the cells in darkness briefly before the next stimulus was administered.

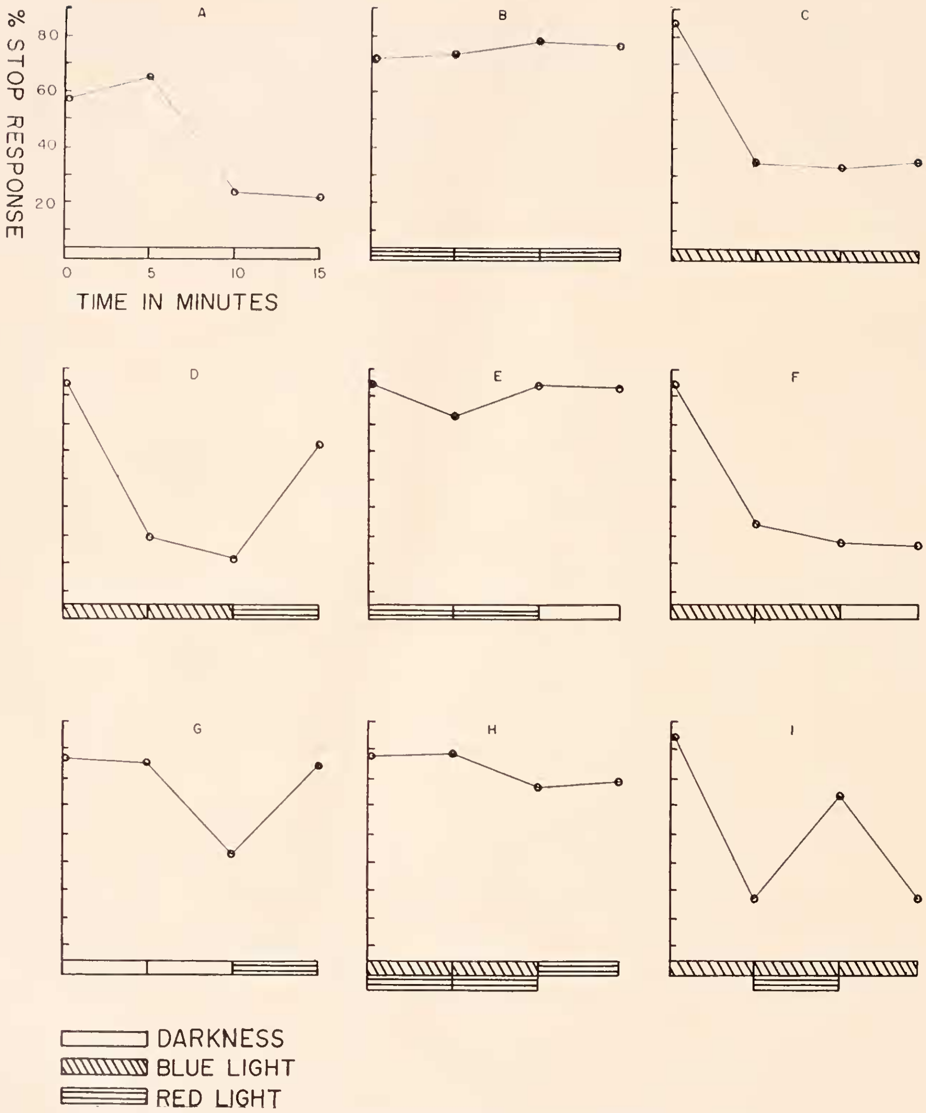

FIGURE 8. The effect of various combinations of red light (600 to 700 mμ at 300 and 280 ft.-c.), blue light (470 mμ at 280 ft.-c., except for 8, F which is 440 mμ at 240 ft.-c.), and darkness on maintenance and regeneration of the stop-response. Each point represents the responses of > 100 individuals.

These experiments were first conducted with the energies of the blue and red sources equal. The series was repeated with unequal intensities, the red being the higher (300 ft.-c.). The results were the same as those of the equal energy series. The combinations of light used in these experiments and the results are shown in Figure 8.

These experiments clearly indicate that red light has a direct effect on the maintenance of the sensitivity of the blue-absorbing system. Cells placed in darkness lose sensitivity over the 15-minute test period (Fig. 8, A), as would be expected, while cells placed in red light during the same test period maintain full sensitivity to stimulation (Fig. 8, B). Cells placed in blue light, with a spectral peak of 470 mμ, lose their sensitivity within the first five minutes rapidly, this presumably being due to light-adaptation (Fig. 8, C).

It is quite possible that photosynthesis is responsible for the "red effect." If so, then blue light of the proper wavelength (440 mμ, 240 ft.-c) should have some maintaining function for the receptor response. To test this, such light was administered for 10 minutes, followed by a 5-minute dark period, this dark period being given to allow the photoreceptor to dark-adapt (Fig. 8, F). As the results indicate, an effect of blue light to maintain the response could not be demonstrated. A similar experiment was performed using red light of the same intensity for 10 minutes, followed by darkness for 5. In this case the response was maintained (Fig. 8, E). This set of experiments does not by any means exclude the possibility that the red effect is due to photosynthesis. It is quite possible that the intensity required for blue light to maintain the stop-response is much higher than that required by red light. Most recently, Diehn and Tollin (1966) have presented some information which suggests that in *Euglena* photosynthesis may be involved in the maintenance of the phototactic process.

In order to determine whether the red system can regenerate the stop-response once it has disappeared, the cells were placed in darkness or blue light for 10 minutes, followed by 5 minutes of red light. As Figures 8, G and 8, D show, the response declined during the first 10 minutes but returned after the cells were in red light for 5 minutes.

Finally we wanted to know what would happen if the cells were given both red and blue light simultaneously. Both light bands were given for 10 minutes, then only red for the last 5. We expected evidence of light-adaptation to blue light by the receptor, as indicated by a low response during the first 10 minutes. Unexpectedly, the response was maintained at the normal high level throughout this time (Fig. 8, H). On the basis of these results we feel that the red process is capable of regenerating the blue photoreceptive system at a rate equal to or faster than the pigment is inactivated at this stimulus intensity. This can be more clearly seen in the last experiment (Fig. 8, I). Here the cells were placed in blue light for the full 15 minutes, but during the second 5 minutes red light was added. As the results indicate, the response level rose, indicating a regeneration of the blue-absorbing system even while the cells were in the presence of constant blue illumination.

VI. *Dark-adaptation in red light*

The dark-adaptation experiment was repeated taking into consideration the effect of red light on the blue photoreceptor. The procedure was the same as that previously described with one change. The sample was placed in red light rather than in darkness following light-adaptation and tested as before. The results of these experiments are shown in Figures 9 and 6, H. The cells dark-adapted faster in red light and also attained a lower threshold.

If one compares the maximum response level attained at each stimulus intensity in darkness (Fig. 5) with similar data obtained in red light (Fig. 9), one sees a decline in the maximum response level in the former. This difference may be attributable to the fact that under the former condition the blue-absorbing photopigment is rapidly bleached by successive stimulation and in the absence of red light cannot be reconstituted rapidly enough to maintain a high response level. In the latter, red light is present, and as has already been demonstrated, the presence of such light allows for regeneration of the blue-absorbing pigment at a rate faster than the sucessive stimuli can degrade it.

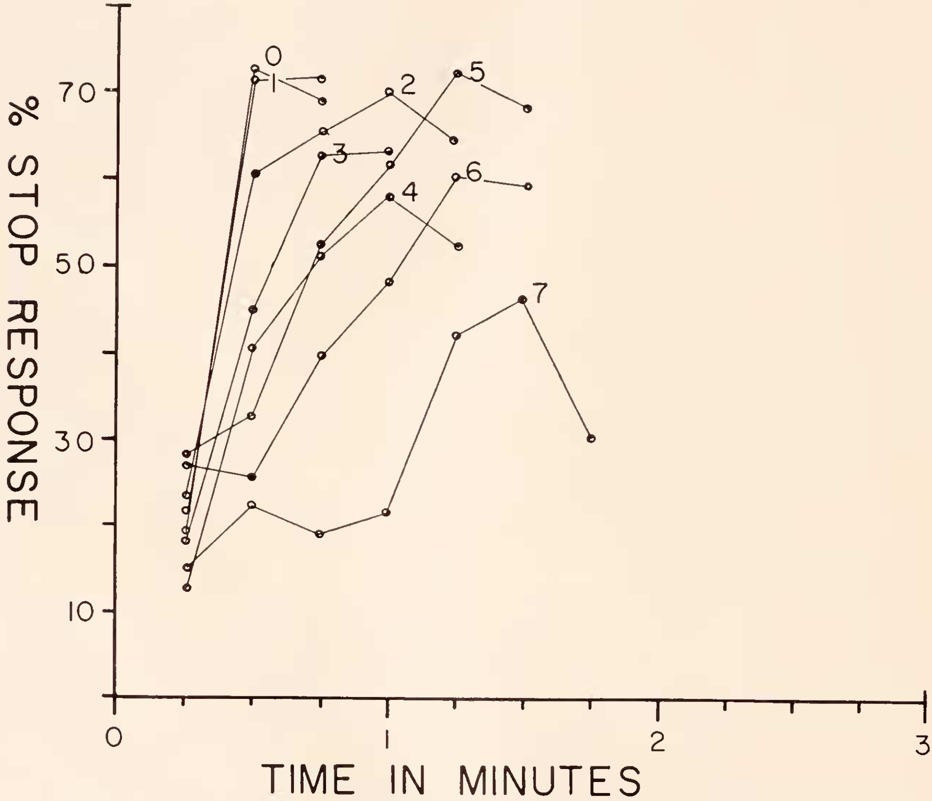

Figure 9. The relation of stimulus intensity to stop-response magnitude when light-adapted cells are placed in red light (600–700 mμ at 300 ft.-c.). The points represent the return to maximum response shown by 100 cells, when these cells are stimulated at different intensities. The numbers represent the different stimulus intensities as given in Figure 5 with two additions: 6 = 77 ft.-c., 7 = 66 ft.-c.

Discussion

On the basis of what is known about photoreception in animals, what can be said concerning the findings presented here? In the animal system, a pigment, rhodopsin, is changed from a photoactive state to a photo-inactive state through a rapid chemical isomerization. The process by which the active state is restored requires several enzymatic steps, and is quite slow (± 30 minutes in man) as demonstrated by the dark-adaptation phenomenon. Most important to this discussion, however, the return to sensitivity of the receptor system requires no photochemical steps. How does this system compare with that of *Gyrodinium*? We assume that a 470 mμ light-absorbing photoreceptor pigment shifts from an active to inactive state when subjected to this wavelength. The shift from active to inactive state triggers processes which ultimately result in a motor response. The entire process, from reception to behavioral act, requires 400 to 600 milliseconds, hence is quite fast. In this respect it resembles the animal system. The difference lies in the regeneration mechanism of photosensitivity. It appears that the regeneration time is short, possibly involving few, if any, enzymatic steps. The key to this short regeneration time lies in the obvious presence of a photochemical step, this being demonstrated by the red light effect. We feel that the red light is a necessary precursor for photo-response regeneration. This assumption is based on four findings. First, it was noticed that *Gyrodinium* would grow, but gave a poor phototactic response, when raised under cold white fluorescent light alone. This light is lacking in the longer wavelengths, emitting predominantly in the blue region of the spectrum. When incandescent light was added, the response increased greatly. Second, we again point out the striking difference attained in dark-adaptation by adding red light during the recovery phase. The third point concerns our ability to dark-adapt the cells in the *absence* of red light. It must be remembered that the intense white light, used to bleach the photoreceptor pigment, must affect both the red- and blue-light-absorbing pigments. If, however, the cells are bleached in blue light alone, the return to sensitivity is not observed when the cells are placed in darkness. Fourth, it is quite clear that spontaneous, rapid, loss of photoresponse sensitivity is observed if the cells are placed in darkness, and that the photoresponse can be maintained or re-established if red light is present.

In order to explain why dark-adaptation was observed in the absence of concurrent exposure to red light, two assumptions concerning the interaction of the red- and blue-light-absorbing systems during bleaching in white light must be made:

1. The blue-absorbing pigment must be inactivated at a rate which exceeds that rate at which it can be reactivated.
2. The red-absorbing system must produce some entity which is stable and produced in excess, if subsequent dark-adaptation in the absence of red light is to occur.

For these two opposing processes to occur, there must be some rate-limiting step present within the inactivation-activation phase. The confirmation of these assumptions lies with biochemical examination of pigment interaction in the cell. Before this is possible, the identity of the pigment or pigments must be determined.

The question remains as to just what the red-blue system represents in terms of the overall photoreceptor mechanism. One idea concerns a two-pigment system, with one pigment supplying energy or some entity necessary for the proper action of the other. It is possible that photosynthesis is involved with photoreception in this manner. Diehn and Tollin (1966) have suggested that photosynthesis is a necessary precursor for phototactic activity in *Euglena*. Their data do not indicate how direct this relationship may be with respect to the photoreceptor. A second possibility exists in a one-pigment system in which response is governed by a shift from a blue- to a red-absorbing state. With the introduction of red light, the pigment returns to the blue, response-active, state. Similarly acting pigments, the phytochromes, do occur in higher plants, and are responsible for the control of flowering and other processes. It is interesting to note that these pigments generally demonstrate peaks of absorption in the blue, red, and far-red regions of the spectrum.

An important point concerns the relationship of this red-blue system to the distribution of a natural population of cells. It has been observed by Hasle (1950) that dinoflagellates undergo a vertical migration, approaching the surface during the day and descending at night. We have observed *Gyrodinium* in extreme numbers at the surface of the water at mid-day. Observations at night indicate that this dense population descends or disperses, for the organisms cannot be found at the surface in the same concentrations. As our data indicate, the photoreceptor machinery loses sensitivity shortly after the onset of darkness. With the loss of sensitivity, movement of the cells becomes undirected relative to light, and they descend under the influence of gravity. As our data further indicate, the receptor sensitivity is recovered if red light is introduced. It is interesting to note that the predominant radiation at dawn exists in the red region of the spectrum. Full photoreceptor sensitivity can be regained, therefore, shortly after dawn, allowing subsequent migration toward the surface to occur. The red system may act as a "cocking" device for the phototactic response, while the response itself is "triggered" by blue light. One must keep in mind that the effects of red light on photoreceptor sensitivity studied here represent only short-term changes in the sensitivity level. Other long-term phenomena such as endogenous rhythmic control of light sensitivity may be present.

It would appear that the vertical distribution of these cells, and possibly of other phytoplankters, is a function of the level of photoreceptor sensitivity, which may change rapidly under varying wavelengths and intensities of illumination.

Summary

1. The effect of blue light on the behavior of *Gyrodinium dorsum* is described. Action spectra for the orientation and "stop" responses are presented.

2. Dark-adaptation curves for the stop-response in darkness and red light are presented.

3. The maintenance effect of red light on these responses is described and a possible two-pigment machinery proposed.

4. The relationship of these phenomena to the vertical distribution of the phytoplankter in nature is discussed.

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